IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: David Morritz De Kretser, et al.

Examiner: Maher M. Haddad

Serial No.: 10/575,049

Art Unit: 1644

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For: A METHOD OF MODULATING INFLAMMATORY RESPONSE BY DOWNREGULATION OF ACTIVIN

Dated: July 7, 2011

Confirmation No.: 5961

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PROFESSOR DAVID MORRITZ DE KRETSER UNDER 37 CFR §1.132

I, David Morritz de Kretser, a citizen of Australia, state and declare the following with respect to the invention described and claimed in U.S. Pat. Ser. No. 10/575,049, entitled "A Method of Modulating Inflammatory Response by Downregulation of Activin."

1. I hold Bachelor of Medicine and Bachelor of Surgery degrees from the University of Melbourne and Doctorate of Medicine degree from Monash University. I am a Fellow of the Royal Australasian College of Physicians, the Australian Academy of Science and the Australian Academy of Technological Sciences and Engineering. I am the founding director of the Monash Institute of Reproduction now known as the Monash Institute of Medical Research. I currently hold the position of Sir John Monash Distinguished Professor at Monash University in Clayton, Victoria, Australia. I am also a co-inventor of the inventions encompassed within the above-referenced patent application.
2. The above-identified patent application describes and claims use of follistatin to treat various nonfibrotic conditions, including systemic inflammation such as septic shock, toxic shock,

septicaemia, and meningitis, as well as lung transplantation, traumatic brain injury, inflammatory bowel disease, severe acute respiratory distress syndrome and asthma. My research isolated follistatin and inhibin and I have studied the role of these proteins and the activins as well as other hormones and growth factors in the context of their role in reproductive processes and in the fields of inflammation and tissue repair since 1975 to the present time. I am recognized internationally as an expert in understanding the roles of these proteins in basic and clinical research.

The '216 Publication

3. The Examiner has rejected all pending claims of the above-referenced patent application, stating that US Patent Publication 2002/0192216 (hereinafter "the '216 publication") as evidenced by van Eyll et al. (*J. of Cell Science*, 117(10):2077-86 (2004)) teaches administering, to a patient in need thereof, a therapeutically effective amount of an inhibitor of a Hedgehog signaling pathway, wherein the inhibitor is follistatin. Several of the therapeutic conditions that are listed as being treatable by inhibiting the hedgehog signaling pathway are inflammatory conditions. The Examiner concludes that since sonic hedgehog (Shh) signaling can be triggered by activin A, inhibiting activin with follistatin would lead to the inhibition of the Shh signaling pathway; thus, the '216 publication and the above-referenced application both use follistatin to treat inflammatory conditions.
4. A review of the literature does not support the disclosure in the specification of the '216 publication that inhibiting Hedgehog signaling can be used to treat inflammation. Several studies indicate that inhibition of Hedgehog signaling actually promotes inflammation.
    - van Dop, et al., *Gastroenterology*, 139:1665-76 (2010) (provided in Appendix A), found that loss of Indian Hedgehog (Ihh) initiates several events that are characteristic of an intestinal wound repair response; however, prolonged loss resulted in progressive inflammation, mucosal damage, and the development of intestinal fibrosis (Conclusion, p. 1665).
    - Zacharias, et al., *Gastroenterology*, 138:2368-77 (2010) (provided in Appendix B), found that inhibition of Hedgehog signaling exacerbates inflammation in gastric tissues. The authors state at page 2370, "[t]ogether, these data indicate that long-term inhibition of

intestinal Hh signaling is associated with crypt hyper-proliferation, progressive villus loss and severe inflammatory disease with phenotypic similarities to human inflammatory conditions." Also, the authors state, "Acute modulation of Hh signals results in changes in inflammatory pathways in intestinal mesenchyme, while chronic inhibition of Hh signaling in adult animals leads to spontaneous intestinal inflammation and death" (Conclusion, p. 2368).

- Nishizawa, et al., *Digestion*, 79:99-108 (2009) (provided in Appendix C), found that hedgehog signaling is down regulated following *H. pylori* infection of the gastrointestinal tract with concurrent enhanced gastric dystrophy, and that following *H. pylori* eradication therapy significant restoration of Shh expression and significant improvement of gastric restructuring was observed (last paragraph, p. 107).
- Lees, et al., *PLoS Med* 5:e239 (2008) (provided in Appendix D), found in human genomic analysis of three Northern European populations, hedgehog signaling genes were down-regulated in patients with diagnosed ulcerative colitis or Crohn's Disease, stating, "[w]e confirm that the HH signaling pathway is down-regulated in colonic inflammation in humans" (Discussion, p. 1769).

5. Other studies have shown that in some systems, hedgehog signaling is upregulated in fibrotic processes, but not in inflammatory processes generally.
   - Stewart, et al., *J. of Pathol.*, 199:488-95 (2003) (provided in Appendix E), investigated the expression of components of the Shh signaling pathway and TGF-β1 in different forms of chronic fibrotic lung disease and studied two experimental models of lung disease—a murine model of ILD (a fibrotic lung disease) induced by intra-tracheal installation of fluoroscein isothiocyanate (FITC) and a murine model of allergic airway inflammation (non-fibrotic) induced by the major dust mite allergen Der p1. On page 490, one of the report titles under the Results section states, "[t]he Shh pathway is up-regulated in murine lung fibrosis but not in Der p1-induced lung inflammation." The authors reported that Shh expression was confined to damaged epithelium at sites of tissue remodeling and fibrosis, but not at sites of normal lung tissue in the models of fibrotic disease; and Shh expression was minimal in the Der p1-induced lung inflammation (nonfibrotic) model (pages 490-2).

6. Because of the complexity of the hedgehog signaling pathway and activin regulation, the impact on Shh expression after treatment with activin A or B or follistatin cannot be predicted.

   - Mfopou and Bowens, *Differentiation*, 76:107-17 (2008) (provided in Appendix F), report that ectopic expression of Shh and subsequent disruption of pancreas development is identified in the presumptive pancreas region of mice bearing mutations in the activin receptor IIB (ActRIIB1/1), further supporting the findings that notochord-derived activin B suppresses Shh expression in the pancreatic region (citing Hebrok, et al, Development, 127:4905-13 (1998) and Kim, et al., Genes and Dev., 14:1866-71 (2000)), which in contrast to a vast majority of other vertebrate organs that are positively influenced by Shh expression (p. 109). On page 113-14, Mfopou, et al. state, "[s]imilarly, another study in mouse ES cells differentiated into EBs and treated for 2 days with a combination of activin A and all-trans retinoid acid showed activation of pancreatic endocrine and exocrine genes in a context of Shh repression."
   - Frandsen, *Biochem Biophys Res Commun*, 362:568-74 (2007) (provided in Appendix G), report that treatment of embryonic bodies derived from human ES cells with activin B leads to a 60-fold increase in sonic hedgehog expression levels (see Figure 4A, p. 573), which could be blocked with activin receptor antagonist SB-431542. Levels of sonic hedgehog after activin A treatment were unremarkable.
   - van Eyll, et al., *J. of Cell Science*, 117(10):2077-86 (2004) (cited by Examiner) report that activin A induced Shh expression, but activin B did not (see Summary p. 2077).

   Thus, as reported by Mfopou, et al., activin inhibits hedgehog signaling in pancreas development and therefore an activin antagonist such as follistatin would stimulate hedgehog signaling. Moreover, Frandsen, et al., report that treatment of ES cells with activin B increases Shh expression levels, where treatment with activin A has no effect on Shh expression levels; yet this result is in contrast with van Eyll, et al., where it was reported that activin A induced Shh in pancreatic explants, but activin B did not. As follistatin would block both activin A and activin B, it is not clear that follistatin could be used to block hedgehog signaling in these systems, and the effect of follistatin on Shh signaling in other systems cannot be predicted.

7. It is my conclusion that the disclosure of the '216 publication—that one can treat a lengthy list of disparate and complex human diseases, including systemic inflammation such as septic shock, toxic shock, septicaemia, and meningitis, as well as lung transplantation, traumatic brain injury, inflammatory bowel disease, severe acute respiratory distress syndrome and asthma with any inhibitor of a hedgehog signaling pathway—is overly simplistic and not supported by studies reported in the literature.
8. It is also my conclusion that one skilled in the art would have to conduct a great amount of experimentation to select 1) follistatin from the myriad of possible inhibitors of the hedgehog signaling pathway disclosed in the '216 publication, and 2) nonfibrotic inflammation from the lengthy list of possible diseases and conditions in the '216 publication to arrive at the working combination of using follistatin to treat nonfibrotic inflammatory conditions. It is also my conclusion that there is a high likelihood that the vast amount of experimentation may not yield a therapeutic response.

The '715 Publication

9. The Examiner has rejected the claims of the above-referenced application under 35 U.S.C. §103(a) as unpatentable over U.S. Patent Publication 2003/0162715 (hereinafter "the '715 publication"). The '715 publication discloses follistatin-like-3 protein to treat disease. The Examiner has agreed with Applicants that follistatin-3 is different from follistatin; however, the Examiner states that those of skill in the art would have had reason to use the follistatin of the instant application as a substitute for the treatment taught in the '715 publication because both follistatin-3 and follistatin are activin antagonists.
10. Follistatin-3 and follistatin are encoded by separate genes and, although they do show some homology, each is a unique protein with distinct roles as demonstrated when the gene for each protein is knocked out. Matzuk, et al. (*Nature*, 374:354-56 (1995), provided in Appendix H)), report that knock-out of the follistatin gene results in the death of all offspring within a few hours after birth. In contrast, disruption of the follistatin-3 gene in mice reported by Mukherjee, et al. (*PNAS USA*, 104:1349-53 (2007), provided in Appendix I) resulted in mice surviving to adulthood.
11. Follistatin has three "follistatin" domains whereas follistatin-3 has only two, lacking the third domain. Although the follistatin domains have some homology, follistatin-3 and

follistatin share only 61.5% amino acid sequence similarity and 43.25% identity. Sidis, et al., *Endocrinology*, 143:1613-24 (2002) (provided in Appendix J) report N-terminal sequencing of follistatin-3 revealed that signal peptide cleavage occurs within exon-1, which is significantly different from follistatin, in which cleavage occurs at the exon/intron boundary (Abstract p. 1613). The most critical difference is that follistatin has a lysine-rich heparin binding sequence in follistatin domain 1 which enables follistatin to bind to heparin sulfate proteoglycans on cell surfaces and targets any follistatin-bound activin to a lyososomal degradation pathway. Follistatin-3 does not have this binding site and therefore cannot initiate the degradation of activin after it is bound. In the same paper, Sidis and colleagues, using different cell models, demonstrate that follistatin-3 is 50-100 fold less potent in neutralizing the effects of endogenously produce activin production whereas it is only 2.4 fold less potent in neutralizing the effects of exogenously added activin A. They conclude "FSRP (follistatin-3) does not have a heparin binding sequence and our studies are a strong indication that the absence of this site prevents cell surface association of FSRP. Thus it is possible that FSRP is unable to achieve the local concentrations in the region of the cell-surface activin receptor that can be achieved by follistatin, thereby leading to FSRP's reduced ability to neutralize endogenous activin." The authors state that this finding suggests that structural differences between follistatin-3 and follistatin may underlie their apparent neutralizing capabilities with respect to exogenous versus endogenous activin. *Id.* Given that inflammatory disorders in humans involve the production of endogenous activin at one or more sites, the absence of the heparin binding site in follistatin-3, will render it ineffective as a therapeutic for these disorders. The teachings in the '715 publication do not provide information to enable substitution of follistatin-3 for follistatin-3. Further, the '715 publication gives no examples either *in vitro* or *in vivo* to exemplify the actions of follistatin-3.

12. The effects of follistatin and follistatin-3 on activin A- or BMP2-mediated gene expression are different depending on the target. Mauer-Satta, et al., *Experimental Cell Research*, 282:110-20 (2003) (provided in Appendix K) explored whether follistatin and follistatin-3 modulate the effects of activin A and BMP2 on human erythropoiesis. They found that follistatin and follistatin-3 block BMP2-and activin A-induced expression of EPO-R and hemoglobin, but the inhibition by follistatin-3 of EPO-R is stronger than that by follistatin

yet only follistatin inhibits activin- and BMP2-induced GATA-2 decreased expression (see Figures 6A and 6B p. 117). The authors conclude that follistatin and follistatin-3 proteins are not redundant and might act on different signaling pathways. *Id.*

13. It has been found in heart failure that follistatin and follistatin-3 have different effects. Lara-Pezzi, et al., *Endocrinology*, 149(11):5822-27 (2008) (provided in Appendix L) examined the expression of follistatin, follistatin-like-1 and follistatin-like-3 in normal and failing heart and after recovery from heart failure. On page 5825, the authors state, "[b]oth FST1 and FSTL3 were up-regulated in cardiac myocytes in the failing heart with expression also evident in endothelial cells and, in the case of FSTL1, in smooth muscle cells. In contrast, FST was not seen in myocytes but was localized to fibroblasts and endothelial cells and did not change in failure." Oshima, et al., *Circulation*, 120:1606-15 (2009) (provided in Appendix M) found that follistatin-3 and activin A are induced after cardiac injury, but that follistatin is not. Injected activin was beneficial in reducing cardiac infarct size after myocardial ischemia/reperfusion injury, and cotreatment with follistatin-3 was detrimental to the positive activin effect (p. 1611-12 and Figure 6C). On page 1614, the authors conclude that follistatin-3 overexpression inhibits myocyte-protective activity of activin A *in vitro*, and that cardiac-deficient follistatin-3 mice display smaller infarcts and less myocyte apoptosis in response to myocardial ischemia/reperfusion injury. Thus, Oshima, et al., teach that follistatin-3 is anti-therapeutic in this setting. Given that follistatin is not modulated by cardiac function, then it is likely follistatin and follistatin-3 are not interchangeable in this system.
14. It is my conclusion that the differences between follistatin and follistatin-3 reported in the literature demonstrate that they cannot be substituted for each other.
15. Moreover, in a recent review of the literature, I did not find one reported study in whole animals or in any system demonstrating that follistatin-3 is effective in treating inflammation.

The '862 Publication

16. The Examiner has rejected all claims under 35 U.S.C. §103(a) as unpatentable over WO 8911862 (hereinafter "the '862 publication"). The Examiner states that the '862 publication teaches that inhibin is useful in wound healing, autoimmune disease, immunodeficiency disease, transplant rejection and infection, and that those of skill in the art would have had

reason to substitute follistatin for the treatment taught in the '862 publication because, like inhibin, follistatin is an activin antagonist.

17. Inhibin and follistatin are different proteins arising from unlinked genes located on separate chromosomes, and comprise very different molecular structures. The inhibin α-subunit gene is located on human chromosome 2q33.34, the βA subunit gene on chromosome 7p13–15 and the βB subunit gene on chromosome 2cen–2q13. The follistatin gene is located on chromosome 5p14. Inhibin is a *bona fide* member of the transforming growth factor-β (TGF-β) superfamily, and is a dimer consisting of an α-subunit crosslinked to a β-subunit (either βA or βB subunits, which are shared with activin) (see Phillips and Woodruff, *Frontiers in neuroendocrinology*, 19:287-322 (2004), provided in Appendix N), whereas follistatin is a single-chain polypeptide with three follistatin domains. Also, as described earlier in the context of follistatin-3, knock-out models of follistatin result in a neonatal lethal with offspring demonstrating defective diaphragms, skeletal defects and growth retardation. Knock-out models of inhibin, on the other hand are born alive and, at 3-4 weeks of age, develop gonadal and adrenal tumours with cachexia and die shortly thereafter (see Matzuk, et al., *PNAS USA*, 91:8817-21 (1994), provided in Appendix O).

18. Further, inhibin and follistatin compete with activin at different cellular levels. Inhibin is an activin receptor competitor that utilizes the TGF-β type III receptor, known as betaglycan, and under some circumstances can sequester TGF-β type II receptors, such as the activin type II receptors (see Bilezikjian, et al, *Reproduction*, 132:207-15 (2006), provided in Appendix P). On the other hand, follistatin binds with high affinitiy to activin subunits (see Phillips and Woodruff, *supra*, Appendix N). Thus, the impact of inhibin and follistatin on activin are fundamentally different, the former being a receptor competitor and the latter being a high affinity binding protein. Finally, inhibin may act as an activin anatagonist in some settings but not in others, whereas follistatin invariably antagonizes and blocks activin actions (see Phillips and Woodruff, *supra*, Appendix N). Literature in the public domain attesting to the former statement includes a recent review in Aleman-Muench and Soldevila (in press (2011), provided in Appendix Q), which states, 'Despite the fact that inhibins have been classically considered as activin antagonists, there is evidence showing that they do not always antagonize activin-mediated functions in several cell types, suggesting the existence of an independent inhibin-mediated signaling pathway.' Further, another publication has

demonstrated that both activin A and inhibin A have similar, as opposed to opposite, actions in fetal thymic organ cultures (Licona-Limon, et al, *Biochemical and biophysical research communications*, 381:229-35 (2009), provided in Appendix R).

19. The only noted effect of inhibin when given *in vivo* and exogenously is to inhibit the reproductive hormone, follicle-stimulating hormone (FSH) (de Kretser, et al., *Human reproduction update*, 8:529-41 (2002), provided in Appendix S). There is no information in the public domain illustrating that inhibin can be administered *in vivo* to effectively modulate inflammatory processes. It should be noted that inhibin treatment in an *in vitro* setting has been shown to have immunomodulatory effects (see, *e.g.*, Broxmeyer, et al., *PNAS USA*, 85:9052-56 (1988), provided in Appendix T). However, one skilled in the art would not take this necessarily to mean that inhibin might be effective in an *in vivo* setting, or that modulation of immune cell lineages and adaptive immunity indicates efficacy in inflammatory processes and innate immunity. In contrast, a number of publications have shown the effectiveness of follistatin administered *in vivo* to block the actions of activin and modulate inflammatory processes (see, *e.g.*, Patella, et al., *Gastointestinal and liver physiology*, 290:G137-144 (2006), provided in Appendix U). The now well-established body of evidence that follistatin modulates inflammation and fibrosis in models of such diseases contrasts with the lack of equivalent findings for that of inhibin.

20. The teachings in the '862 publication are also confusing since, as described above, inhibin may act as an activin antagonist in some settings and as an agonist in other settings. Therefore, immunizing against inhibin, and therefore blocking its "activin antagonism," may have an action completely the opposite of follistatin, which blocks the actions of activin in every circumstance. In addition, I submit that the teachings of the '862 publication have not been substantiated by research in the years since the filing of the application that led to the '862 publication. For instance, on page 5, lines 13-15, the '862 publication teaches that, "[A]ctivin is suitable as 1) and agent for the treatment of autoimmune diseases and for inhibition of transplantation rejection responses...." I submit that this statement has been refuted insofar as it teaches that activin is suitable for inhibiting transplantation rejection responses. The utility of activins for inhibiting transplantation rejection is not supported by the literature, which in fact teaches that an activin antagonist, in this case follistatin, actually is beneficial in transplantation responses (see Benabdullah, et al., Cell transplantation,

18:709-18 (2009), provided in Appendix V) and Kanamoto, et al., Digestive diseases and sciences, 56:1075-81 (2011), provided in Appendix W)).

21. Moreover, one skilled in the art would be aware that results in an embryonic fibroblast cell line—as exemplified in Example 2—may have limited applicability to other fibrogenic processes. Example 2 in the '862 publication presents results in an *in vitro* setting only, and direct applicability of these studies to complex *in vivo* environments cannot be automatically assumed. In point of fact, it is known that fibrogenic processes, such as wound healing and scarring, are altered in fetal life as skin wounds to fetuses heal without scarring (see Larson, et al., *Plastic and reconstructive surgery*, 126:1172-80 (2010), provided at Appendix X). Therefore, an *in vitro* result in an embryonic fibroblast lineage cannot be assumed to translate to fibrogenic processes in postnatal life, and the teaching is of limited or no value with regard to *in vivo* therapeutic uses of inhibin—much less follistatin—in postnatal animals.
22. The failure of inhibin to block the proliferative actions of activin A on 3T3 cell proliferation is again a further example of its action being used to predict the actions of follistatin which effectively blocks all the known actions of activin A. In part this may result from the fact that fibrosis is a complex process controlled by multiple entities which cannot be modeled *in vitro* where a single agent acts to stimulate proliferation of a fibroblast cell line. In addition to activin A, the fibroblast can respond to Transforming Growth Factor-β , platelet derived growth factor, endothelin, angiotensin and connective tissue growth factor (Ask, et al, *Internat J Biochem and Cell Biol*, 40:484-95 (2008), provided at Appendix Y; Ross, et al, *Am J Respir Cell Mol Biol*, 42:16-20 (2010), provided at Appendix Z; Xu, et al, *Arthritis & Rheum*, 56:4189-94 (2007), provided at Appendix ZA; Karger, et al, *Cell signaling*, 20:1865-72 (2008), provided at Appendix ZB; Osterreicher et al, *Hepatology*, 50, 929-39 (2009), provided at Appendix ZC; Zhong et al, *Hypertension* 57, 314-22 (2011), provided at Appendix ZD). Proof of an *in vivo* action on fibrosis is critical to establish the therapeutic potential of any anti-fibrotic agent.
23. Throughout the teachings of the '862 publication the term "tissue proliferation" is used despite the only evidence presented being the proliferation of a single cell type. A tissue is the composite of many cell types and claims to tissue proliferation cannot be made on the basis of the example given. In fact the two examples show that activin has differing actions on proliferation on the 3T3 and thymocyte cell types.

I declare that all statements made herein based on my own knowledge are true and that all statements based on information and belief are believed to be true; and further that the statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issued therefrom.

Dated: 8th July 2011

Professor David Morritz de Kretser